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Susceptibility of Ponderosa Pine to Western Gall Rust Within the Middle Columbia River System

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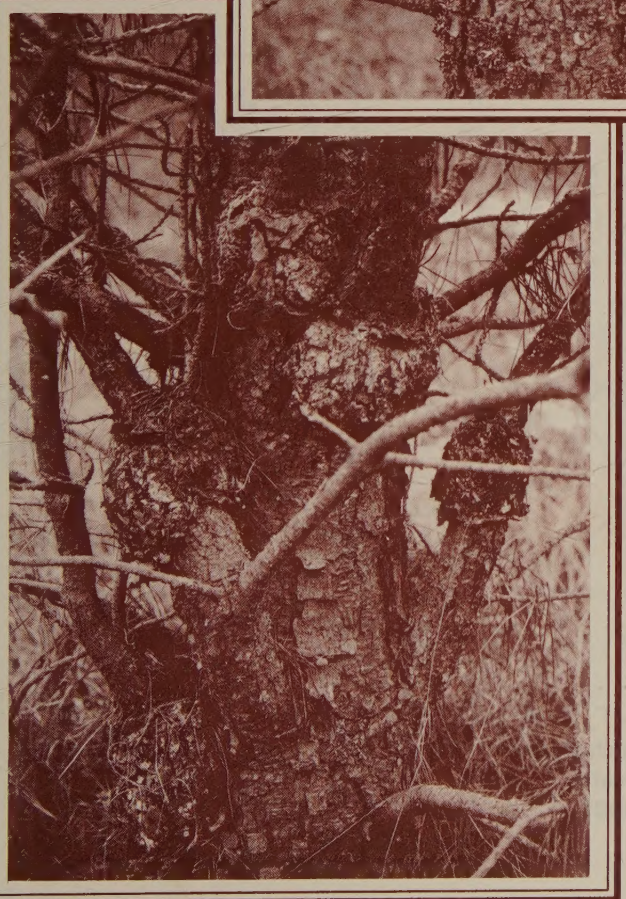

THE AUTHOR

R. J. HOFF is principal plant geneticist with the Intermountain Research Station's Forestry Sciences Laboratory in Moscow, ID. He received a B.A. degree in biology from Western Washington State University and a Ph.D. in botany from Washington State University.

RESEARCH SUMMARY

Ponderosa pine from the middle Columbia River system proved to be highly resistant to western gall rust. Only 28 percent of the seedlings that were inoculated at the beginning of their second growth year were infected 39 months after artificial inoculation. Three major resistant reactions were observed: trees that expressed no symptoms, trees that had visible bark reactions, and trees in which the fungus causing the initial infection later died.

The degree of infection increased with elevation, with 15 percent of the variation accounted for. A stepwise multiple regression used a combination of elevation, azimuth, distance from inoculum source, and southwest departure to account for 26 percent of the variation. This analysis also indicated that the populations that were nearest to the inoculum source were most susceptible.

My conclusions were: (1) Most of the variation in susceptibility to western gall rust was random. (2) There was a moderate association with elevation and geographic area. (3) Three distinct resistance reactions were operating.

This information should be useful to forest managers in selecting stands for collecting seed and for selecting superior seed trees for reforestation and tree improvement.

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Susceptibility of Ponderosa Pine to Western Gall Rust Within the Middle Columbia River System

R. J. Hoff

INTRODUCTION

Western gall rust caused by *Endocronartium harknessii* is a relatively minor disease of northern populations of ponderosa pine (*Pinus ponderosa* var. *ponderosa*). Most damage is to young trees and galls are frequently confined to branches (Leaphart 1955; Peterson 1959).

Thomas and others (1984) reported that ponderosa pine from Idaho, eastern Washington and Oregon, and central and western Montana were most resistant, and that populations from the Colorado plains and the Southern Rockies were most susceptible to gall rust. A more detailed study of central Idaho populations, using artificial inoculation with high spore density, revealed many highly susceptible populations (Hoff 1986). Infection among populations ranged from 65 to 95 percent.

The purpose of this paper was to assess variation patterns in susceptibility to western gall rust in populations of ponderosa pine from northeastern Washington, northern Idaho, and western Montana.

MATERIALS AND METHODS

Seeds from 125 populations from northeastern Washington, northern Idaho, and Montana west of the Continental Divide, and covering the elevation range of the species, were included in this test (fig. 1). Most stands were represented by an equal volume of seed from 10 randomly chosen trees. Some collections were from several squirrel caches. Diversity was assured in the squirrel cache collections by selecting cones with a variety of morphologies, sizes, and colors. The seeds were sown in containers (8 in^3) and grown and overwintered in a shadehouse at the Forestry Sciences Laboratory at Moscow, ID (lat. 46°44' N., long. 1170°0'W., elevation 2,650 ft). The seedlings were watered and lightly fertilized, with the objective of producing seedlings that were not stressed but without accelerating their growth.

The experimental design was: 125 populations, 10 seedlings per population per replication, three replications. Seedlings were grown in row plots. Total seedlings were 1,250 per replication and 3,750 for the test.

Figure 1—Number and locations of populations of ponderosa pine stands that were included in this test. Letters A to D locate elevation clines presented in figure 4.

In May the seedlings were moved into a mist chamber in preparation for inoculation. The seedlings were just beginning their second growing season, and needles were just beginning to break out of the fascicle sheaths. Mist nozzles were operated to provide a constant visible fog throughout the chamber. Twenty-four hours later, the stems of the seedlings were wet and allowed to sit for 1 hour to let excess moisture drain. The seedlings were then inoculated.

Fresh inoculum was collected from at least 50 galls from a young ponderosa pine stand located at the Lone Mountain Tree Improvement site (lat. 47°54' N., 116°49' W., elevation 2,488 ft) about 25 miles north of Coeur d'Alene, ID. Inoculation was accomplished on May 30, 1984, by blowing spores over the seedlings using an air-sprayer adapted from an Erlenmeyer flask. Each replication was inoculated separately with a mixture of 1.2g of aeciospores and 7g of talc. This gave a spore-to-seedling ratio of about 20,000 to 1. The seedlings were kept in the mist chamber for an additional 72 hours making sure that a visible fog was maintained. The mist nozzles were then turned off and the seedlings allowed to slowly dry. When dry, they were moved to a shadehouse. After a couple of weeks of acclimation, they were moved to a nursery at Priest River Experimental Forest (lat. 48°21' N., long. 116°52' W., elevation 2,400 ft), where they were planted at a 6- by 6-inch spacing in beds 30 ft wide. The 10 seedlings for each provenance in each replication were planted in two adjacent rows of five trees each. Planting dates were June 19 and 20, 1984.

Inspections for symptoms of infection were made for each seedling at 3 months, 15 months, 27 months, and 39 months after inoculation. Data were tallied for the presence of galls and reactions on the stem indicative of defense reactions. The following variables were used to assess variation among populations:

1. Infected. Trees with galls at 39 months.
2. Resistance. Trees with no galls at 39 months. These were placed into three categories of resistance:
   a. No reactions. Trees that did not have galls or bark reactions.
   b. Bark reactions. Trees that did not have galls but had visible reactions on the stem indicative of defense reactions.
   c. Gall death. Trees that had galls at 3, 15, or 27 months after inoculation for which the gall was dead at 39 months.
3. Rate of gall appearance. Percentage of trees with galls at 39 months that were visible at 3 months.
4. Rust-killed trees. Trees with galls that died by 39 months.

Analysis of variance was used for each variable to determine significant differences among stands. Simple correlations were performed for the rust variables with 3-year-old height, needle length, percentage dead due to drought, and initiation of bud growth in a greenhouse. The growth and drought data were from Rehfeldt (1986) from different seedlings but from the same populations and same seed collection.

Simple and multiple regression models were used to relate the rust variables to elevation and geographic location of the seed source. The independent variables were elevation, latitude, longitude, northwest departure, southwest departure, azimuth from the inoculum source, and distance from the inoculum source. Northwest (latitude x longitude) and southwest (1/latitude x longitude) departures were derived by rotation of the grid of latitude and longitude by 45 degrees. Multiple regression was used when simple regression analysis of the rust variables were significant. Squares of the above independent variables were added to accommodate the possibility of nonlinear patterns of variation. Therefore, 14 independent variables were included in a stepwise regression for maximizing R^2 (SAS 1982). In addition, the geographic variables were nested within four geographic regions: northeastern Washington, most of Idaho, northwestern Montana including the most northerly stands in Idaho, and middle to southwestern Montana (fig. 1).

RESULTS

The analyses detected differences among populations for the degree of infection and in the kinds of resistance reactions (table 1). Most rust factors varied randomly with the environment; the degree of infection was the only factor that was related to elevation or geographic origin of the seed.

The average level of trees with galls 39 months after inoculation was 28 percent; populations varied from 3 to 63 percent (table 1 and fig. 2). Three major categories of resistance were evident: (1) trees that expressed no symptoms—38 percent; (2) trees that expressed bark reactions—52 percent; and (3) trees in which the galls died—10 percent. Differences among populations for rate of gall appearance and for death due to rust were high but not significant.

There were no strong associations between the rust factors and the environmental factors (table 2). The R^2 for level of susceptibility and elevation was 0.15, the highest single association. Using all environmental factors with the level of infection, the R^2 for the best fit stepwise multiple regression model was 0.26. Figure 3 shows geographic lines at a constant elevation (average elevation for all stands). The distance between contour lines equals $\frac{1}{2}[lsd(0.2)]$. Thus distance equivalent to two contour lines differs by a probability of about 0.2. When comparing populations from the same elevation, populations from the west central area—the area near the origin of the rust inoculum—displayed the highest level of infection. From here, the level of infection decreased in all directions.

Figure 4 shows the association between elevation of the seed source and the level of infection. The elevation clines are keyed to the four geographic areas shown to figure 1. And they all indicate that the degree of infection increases as elevation of seed source increases, although the relationship tails off at high elevation.

Table 1—Means, range, and analyses of variance of symptoms of infection among provenances of ponderosa pine 39 months after artificial inoculation with rust

Symptom of infection	Analysis of variance					
	x	Range	df	MS	F	PR>F
	-- *Percent* --		*No.*	*Percent*		
Infection (trees with galls)	28	3-63	124	[1]0.090	2.11	0.0001
Resistant (trees without galls)	72	37-97	124	[1].102	2.04	.0001
Categories of resistance:						
No galls or bark reactions[2]	38	8-76	124	[1].110	1.42	.0106
Bark reactions only[2]	52	13-89	124	[1].093	1.30	.0446
Gall death[2]	10	0-36	124	[3].008	1.35	.0230
Trees with galls or bark reactions	72	28-93	124	[1].102	1.51	.0035
Rate of gall appearance[4]	35	0-78	124	[1].291	1.11	.2438
Rust-killed trees[5]	8	0-44	124	[3].012	1.00	.4955

[1]Transformed to arcsin $\sqrt{x}$.

[2]Percentage resistance category is of total resistance.

[3]Transformed to $\sqrt{x + 0.5}$.

[4]Rate of appearance determined by number of galls 3 months after inoculation/by number galls 39 months after inoculation.

[5]Data from galled trees only.

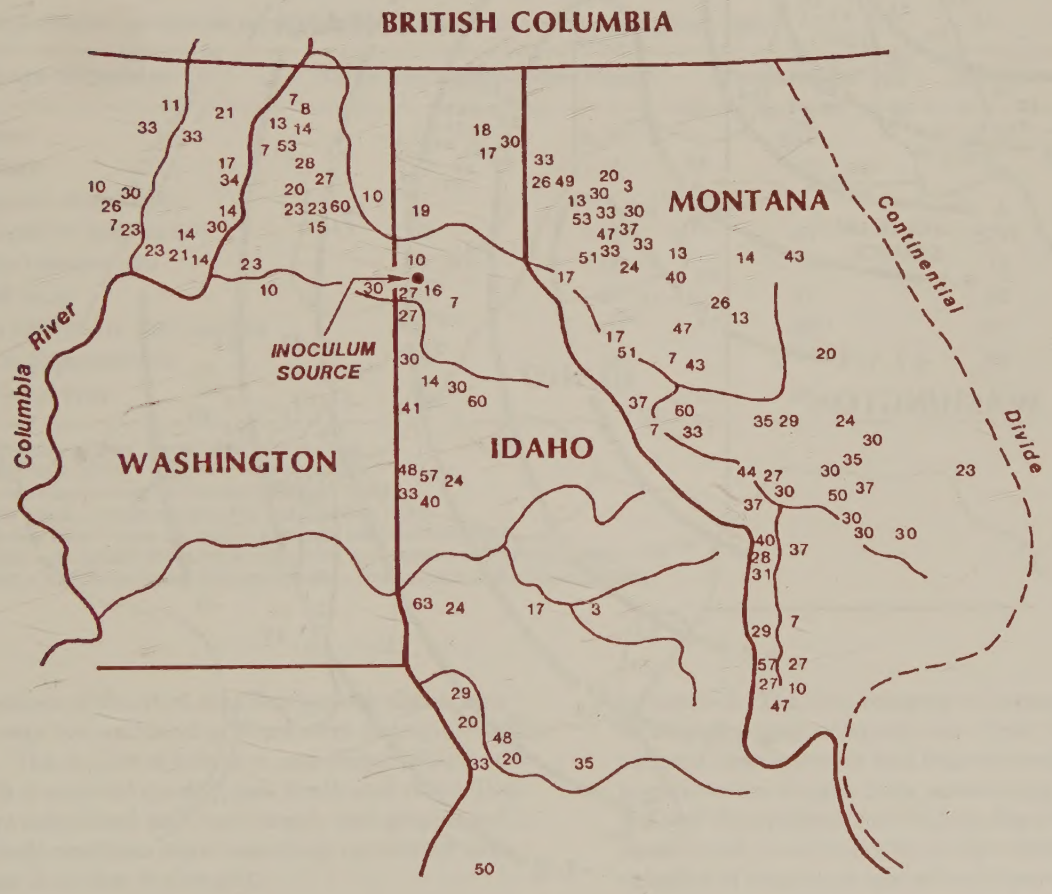

Figure 2—Level of infection of populations of ponderosa pine to gall rust. Numbers represent actual percentage of trees with galls for each population.

Table 2—R^2s between rust factors and various environmental factors[1]

Symptom of infection	Environmental factors						
	El	Lat	Long	NW	SW	AZ	Dist
Infection	0.15**	0.08**	0.05**	0.09**	0.02	0.05**	0.02
Resistant	.15**	.08**	.05**	.09**	.02	.06**	.02
Categories of resistance:							
No galls or bark reactions	0	0	0	0	0	0	0
Bark reactions only	.01	.02	.01	.02	.01	.01	0
Gall death	.01	.02	.02	.02	0	.02	.01
Trees with galls or bark reactions	0	0	0	0	0	0	0
Rate of gall appearance	0	0	.02	.01	0	.01	0
Rust-killed trees	0	.01	0	0	.02	0	0

**Significantly different at 1 percent.

[1]El = elevation, Lat = latitude, Long = longitude, NW = northwest departure, SW = southwest departure, Az = azimuth, Dist = distance from the inoculum source in miles.

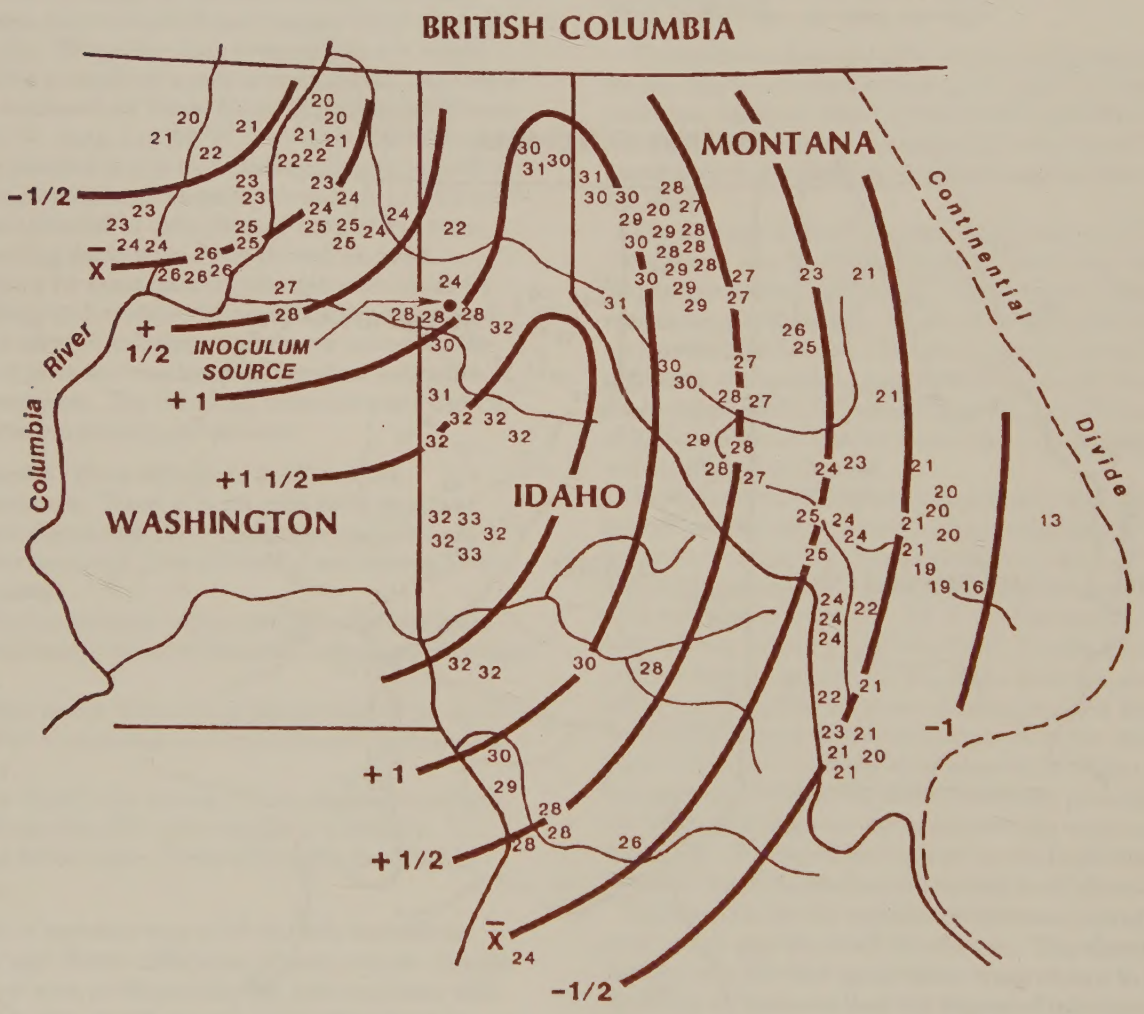

Figure 3—Level of infection of populations of ponderosa pine to gall rust. Numbers represent predicted percentage at the mean elevation of all populations (3,525 ft). Contour lines represent lines of equal performance. The interval between contour lines equals $^1/_2[Isd(0.2)]$ and lines represent positive or negative deviations from the mean value of all populations.

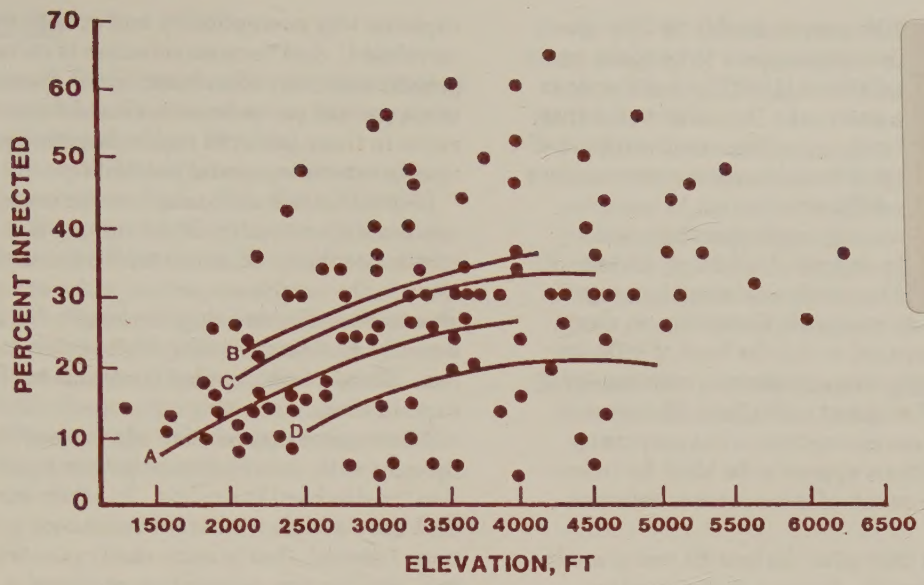

Figure 4—Actual percentage infection plotted against elevations. Lines A to D represent elevation clines for the geographic areas indicated in figure 1.

Table 3—Simple correlations among population means for rust data and growth data

Symptom of infection	Three-year height[1]	Leaf length[2]	Percent dead[3]	Elong[4]	Start[5]
Infection	–0.19*	0.10	0.12	–0.11	–0.07
Resistant	.19*	–.10	.12	.11	.07
Categories of resistance:					
No galls or bark reaction	.03	–.03	.24**	–.16	.03
Bark reactions only	.01	–.09	–.16	.14	–.02
Gall death	.04	.21*	.17	.03	–.01
Trees with galls or bark reactions	–.03	.07	–.25**	.07	.01
Rate of gall appearance	.06	.15	0	.05	–.17
Rust–killed trees	.06	.19*	–.05	.14	.04

*Significant at the 5 percent level of probability.
**Significant at the 1 percent level of probability.
[1]3-year height = height in the field (Rehfeldt 1986).
[2]Leaf length = length of leaf in the field (Rehfeldt 1986).
[3]Percent dead = trees that died in a drought test (Rehfeldt 1986).
[4]Elong = elongation of second year's growth in a greenhouse (Rehfeldt 1986).
[5]Start = initiation of growth in a greenhouse = time to reach 2 mm (Rehfeldt 1986).

Correlations of the rust data and growth characters were all very low and most of them were nonsignificant (table 3). The degree of infection was negatively correlated with 3-year-old growth; gall death and rust-killed trees were correlated with leaf length; and presence of galls or bark reactions were negatively correlated with percentage dead due to drought.

DISCUSSION

The general level of resistance of this collection of ponderosa pine populations to western gall rust was high; nonetheless, there were several populations that were highly susceptible. Three resistance categories were recognized. The first category of resistance was expressed as trees that had no symptoms. Such trees had either escaped inoculation or had defense mechanisms that prevented the fungus from penetrating the cuticle or cell walls of the epidermis or that confined the infection after penetration, so as to not be easily visible. The second category of resistance comprised trees with easily visible stem reactions. Whatever caused these reactions, possibly chemicals toxic to both host and fungus cells, resulted in the death of the fungus. The third category of resistance was expressed as typical galls at one of the first three inspections, but that were dead at the fourth inspection. This condition was signaled by a patch of necrotic tissue surrounding and encompassing the gall.

These three kinds of reactions were similar to those described by Hoff (1986). They also appear to be of the same type reported by Allen and others (1988) for resistance in lodgepole pine to western gall rust. Two other resistance reactions, namely rate of gall appearance and variation of rust-killed trees, were highly variable among provenances but were not statistically different.

Much of the variation among populations appeared to be random. But, for the degree of infection, variation among populations could be partly explained by geography. The R^2 for elevation was 0.15. Generally, as elevation of populations increased so did the level of infection; however, the relationship was curvilinear, with the level of infection leveling off at about 4,000 feet. That low-elevation sources are less susceptible is not surprising because weather conditions appear to be ideal for infection, with the probable result of more intense selection for resistance.

The multiregression that gave the best fit was a combination of elevation, azimuth, distance from inoculum source, and southwest departure. These accounted for 26 percent of the variation in infection. Most surprising was that the predicted level of infection was higher near the inoculum source, and then decreased in all directions with distance from that point. If resistance was closely tuned to the genetic variation of the fungus, the opposite pattern would be expected. Possibly variation within the rust is absent or very small and the differences among the populations, as indicated by the model, represent the interaction of the environmental conditions and genetics of the host. On the other hand, Powers and Matthews (1980) felt that the fungus has adapted to the host and consequently has produced races that are more virulent in the local sites. I find this explanation easier to accept with a system like loblolly pine; namely a fusiform rust that has been excessively disturbed, than with a system like ponderosa pine; characterized by western gall rust, which has had comparatively little disturbance.

The small but significant negative correlation of the level of infection and 3-year height is consistent with the association of growth and elevation, namely low-elevation populations grow faster and are less susceptible to western gall rust and high-elevation populations grow slower and are more susceptible. The clines in Rehfeldt's (1986) figure 2 and figure 4 in this paper show these relationships. But the association between infection and geographic pattern as predicted by the model and growth was not consistent because the fastest growing populations were from the west-central portion of the sample area (Rehfeldt 1986). These populations were the most susceptible, based on the predicted value, and from here growth and of susceptibility both decrease outwards. Rehfeldt's figure 3 on the geographic growth pattern and the geographic pattern of rust susceptibility (fig. 4) are almost identical. Thus there are two contrasting clines: (1) With geographic area, susceptibility decreasing with slower growth outwards in all directions from the inoculum source and (2) susceptibility increasing with slower growth due to an increase of elevation. I cannot explain this difference; however, the higher elevation effect, as shown by the much larger simple correlations, possibly explains why susceptibility and growth are negatively correlated. And because infection is correlated with growth when elevation is set to the mean of the populations, growth per se is not a causal factor in the differences in these patterns and is therefore possibly due mostly to environmental patterns.

Likewise it is hard to explain the association of long needles with mortality of the fungus and mortality of rust-killed trees. Rehfeldt (1986) found that the populations in the northwest portion of the sample area had the shortest needles and that the length increased in a southeasterly direction. Possibly these relationships are spurious. If real, more detailed work will have to be done to explain them.

The negative correlation of the trees that died of drought with stem reactions (either a gall or bark reaction) is also hard to explain. In other words, populations that have a higher inherent resistance to drought were more infected, that is more easily penetrated by the fungus, whether the penetration produced a gall or was repulsed by a bark reaction. This could possibly be due to chemical aspects of the stem, fatty acids, or higher moisture content of drought resistant cells—the fungus has only to penetrate the cuticle to get into a "good" environment. On the other hand, the root structure of drought-resistant provenances may produce moisture conditions in the stem that are best suited for penetration by the fungus. Answers will also have to wait for more detailed analyses of the penetration process and the environmental requirements of the process.

A major difficulty with artificial inoculation is that the trees are inoculated at one point in time. This coupled with the variation of growth initiation and rate and amount of growth among populations could lead to susceptibility patterns related to periodicity instead of inherent resistance of each population. But correlations of the growth data, either from the field or greenhouse, with total stem symptoms (galls plus bark reactions) were all very low and nonsignificant, indicating no relationship of initial infection level with growth characters for populations. I assumed therefore that all the populations had an equal chance of becoming infected during the period of inoculation and that differences would be due to the inherent genetic nature of the population in terms of susceptibility to gall rust and not to its periodicity.

MANAGEMENT IMPLICATIONS

In terms of tree improvement, there appears to be little danger in moving seed within the middle Columbia River system unless a particularly susceptible stand unknowingly is chosen for the source of seed. Thus, it is important for forest managers to closely inspect stands prior to seed collection to determine the level of susceptibility to western gall rust. Further, because some individuals are highly susceptible, care must also be taken in selecting seed trees for natural regeneration, or in selecting trees to include in tree improvement programs.

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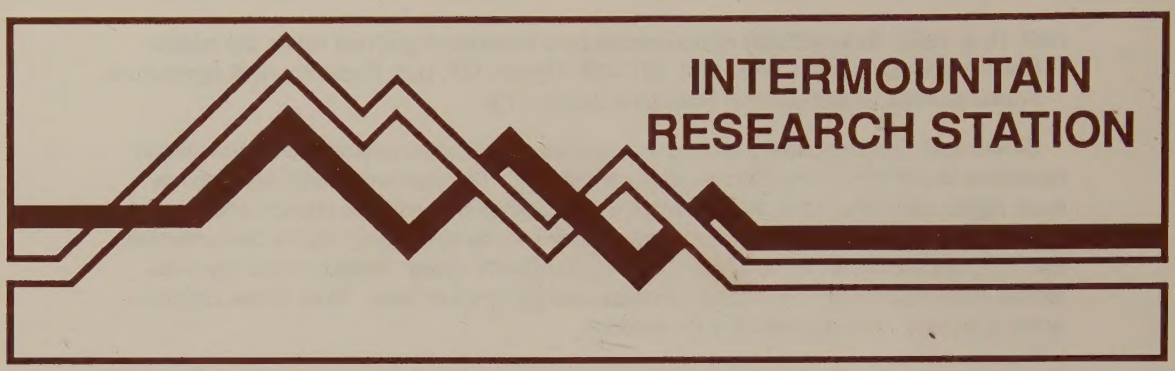
Differences in susceptibility among provenances of ponderosa pine were observed in response to artificial inoculation by western gall rust. The northern inland populations were highly resistant. Only 28 percent of the seedlings had galls 39 months after inoculation. Three major resistant reactions were observed: no symptoms, visible bark reactions, and fungus causing an initial infection but subsequently dying. Relationships were detected between infection level and elevation and geographic area. Most of the variation among provenances appeared to be random.

KEYWORDS: rust resistance, disease resistance, provenance test, artificial inoculation



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